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# *Defluorination of Methoxyflurane during Anesthesia:*

## *Comparison of Man with Other Species*

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Human plasma and urinary inorganic fluoride concentrations were elevated within 30 minutes after initiation of methoxyflurane administration and increased progressively during anesthesia. To define an animal model for studies of the effect of such rapid methoxyflurane catabolism to fluoride ion, defluorination was evaluated in calves, monkeys, dogs, rabbits, guinea pigs, rats, and mice. All animals defluorinated methoxyflurane. (Key words: Methoxyflurane; Defluorination; Comparative metabolism.)

Since 1964, two years after its clinical introduction, there have been reports of methoxyflurane-induced renal dysfunction.[1-12] In 1970 it was suggested that fluoride, a metabolite of methoxyflurane, is the nephrotoxic substance.[8] Renal effects of fluoride in rats,[13] dogs,[14] and man[15] suggest that this ion causes toxicity similar to that caused by methoxyflurane. Correlation between serum fluoride levels and renal dysfunction after methoxyflurane anesthesia has been reported.[11] However, no study to date has established the temporal relationship of methoxyflurane administration and serum fluoride increases to onset of renal dysfunction,[16] although polyuria in the recovery room,[10] excess urinary inorganic fluoride 2 to 4½ hours after exposure,[17] and increased serum fluoride ion on the first postoperative day[8, 11] have been reported. The above data suggest that fluoride is the toxic substance, but methoxyflurane and its other metabolites have not been eliminated as causative factors in renal dysfunction. If fluoride is the primary renal toxin involved, the change in renal function should be proportionate, quantitatively and temporally, to the increase in serum and/or urinary fluoride during methoxyflurane administration.

The present study was conducted to investigate defluorination of methoxyflurane in human and animal species with the intent of defining a model system of defluorination for further evaluation of fluoride as a causative factor of the post-methoxyflurane renal changes.

## Materials and Methods

Comparative defluorination of methoxyflurane was investigated by measuring plasma and urinary fluoride ion concentrations in man,

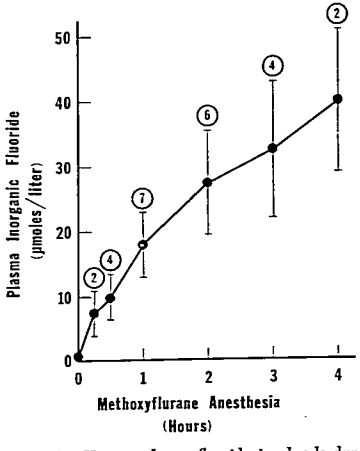


Fig. 1. Human plasma fluoride ion levels during methoxyflurane anesthesia. Numbers of samples are indicated in circles; vertical lines represent ±SD of the mean. Fluoride concentrations prior to anesthesia (n = 6) ranged from <1 to 3 μmol/l. Methoxyflurane concentrations averaged 1.0 per cent during induction, 0.5 per cent at 30 minutes, and decreased progressively during anesthesia.

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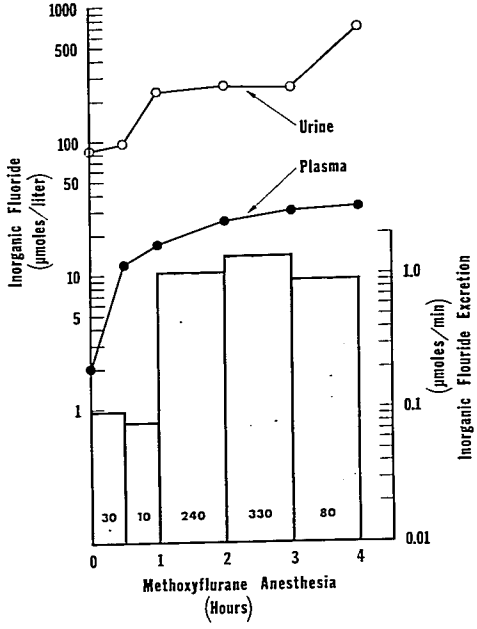


FIG. 2. Simultaneous fluoride ion concentrations (left ordinate) and fluoride excretion (shaded bars, right ordinate) in human plasma and urine during methoxyflurane anesthesia. Volumes of urine (ml) of the respective collection intervals are noted in the shaded bars. Urinary osmolalities for hours 2, 3, and 4 were 137, 42, and 207 milliosmols, respectively; the control value was 1,162 milliosmols.

Swiss Webster mice, Sprague-Dawley rats, Hartley guinea pigs, New Zealand rabbits, mongrel dogs, and monkeys (*Macacus arctoides*).

### HUMAN SUBJECTS

Seventeen patients (nine men and eight women) who received methoxyflurane anesthesia for various surgical procedures were studied. Urine was collected intraoperatively by catheterization, and from early morning voidings on the first three postoperative days; plasma was collected during anesthesia only. Control urine and plasma samples were obtained prior to methoxyflurane anesthesia and from patients who received halothane anesthesia.

### ANESTHESIA CHAMBER

Animals of one species were placed in a glove-box chamber (volume = 230 liters) with attached interchange compartment.‡ A vaporizer§ with an oxygen input of 15 l/min delivered to the chamber at both sides near the bottom an oxygen–methoxyflurane mixture which was exhausted from one point at the top through a motor-driven fan. The chamber and gas-delivery tubes were glass or metal, and the enclosure was maintained at atmospheric pressure with continuous monitoring. Attached gloves were of milled butyl rubber. Temperatures during anesthesia ranged from 23 to 26 C. Vaporizer calibration and concentrations of methoxyflurane in the chamber were measured with a gas chromatograph as previously described.[18]

‡ Kewaunee Scientific Equipment, Adrian, Michigan.

§ Pentomatic, The Foregger Co., Smithtown, New York.

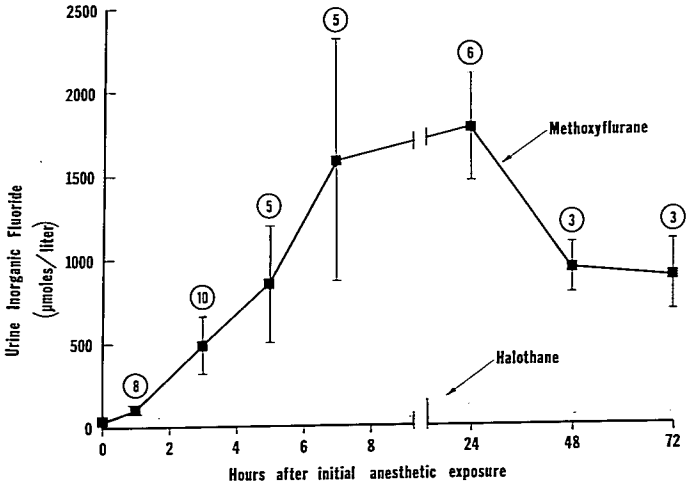


FIG. 3. Fluoride concentrations in samples of urine obtained within two-hour intervals during anesthesia (2–7 hours' duration) and in the recovery room, plus collections on the first three postoperative mornings (mean ±SE). Numbers of samples are shown in circles. The shaded areas represent the range of 50 control samples taken from eight patients (five men and three women) before, during, and after halothane anesthesia. Methoxyflurane concentrations averaged 1.2 per cent during induction, 0.7 per cent at 30 minutes, and decreased progressively during anesthesia.

### ANESTHESIA OF ANIMALS

Mice, rats, guinea pigs, and rabbits were anesthetized in groups of ten, five animals of each sex per species. Anesthesia was initiated at the maximum vaporizer setting, which produced chamber concentrations of 1–2 per cent methoxyflurane. The vaporizer was then adjusted to the minimum setting necessary to maintain all animals unresponsive to a painful stimulus, *i.e.*, extremities were pinched at frequent intervals with toothed forceps. After half an hour of exposure, 0.9 per cent sodium chloride solution (equal to 1.3 per cent of body weight) was injected intraperitoneally to stimulate urine production.

The larger species, *i.e.*, dogs and monkeys, were anesthetized in groups of two, one of each sex. Regulation of anesthesia and administration of saline solution were carried out as with the smaller animals.

In addition, two male calves were anesthetized with methoxyflurane utilizing conventional anesthetic equipment. Urine and plasma, including a pre-methoxyflurane sample, were obtained at intervals.

### SAMPLE COLLECTION

After three hours of exposure to anesthetic concentrations of methoxyflurane, the animals were removed from the chamber for collection of plasma and urine. Blood samples were drawn with heparinized plastic syringes and centrifuged. Urine was collected by bladder puncture. Both plasma and urine were frozen in plastic tubes and analyzed within 15 days. A sample of the injected saline solution was also collected for fluoride analysis.

Control samples were obtained during sodium pentobarbital anesthesia in rats (50 mg/kg), guinea pigs (35 mg/kg), rabbits (40

mg/kg), and dogs (30 mg/kg). Mice were anesthetized with ether and monkeys with ketamine HCl (90–105 mg/kg, total dose). Plasma and urine samples were collected 2.5 hours after intraperitoneal injection of 0.9 per cent saline solution equal to 1.3 per cent of body weight.

Water and food were provided *ad libitum* until the initiation of anesthesia.

### Fluoride Analysis

Fluoride ion levels were determined with a fluoride ion electrode, expanded-scale *p*H meter, and a ceramic junction calomel standard reference electrode.¶ Plasma fluoride was measured ten minutes after the addition of 0.1 ml of acetate buffer to 1 ml of plasma according to the method of Fry and Taves.[19]

A urinary fluoride analysis was developed as follows: In a plastic beaker 4 ml of urine were adjusted to *p*H 5–7 with one or two drops of 1 N HCl. The fluoride electrode potential was determined, and conductance measured.** The activity coefficient of fluoride ion was calculated after conversion of conductivity to equivalent NaCl ionic strength.[20] Fluoride ion activity was interpolated from a standard curve (electrode potential *vs.* activity) and divided by the activity coefficient to give fluoride ion concentration. This method of urinary fluoride ion analysis was accurate to within 7 per cent in solutions of $10^{-6}$–$10^{-2}$ molar fluoride and 0.05–0.52 molar ionic strength.

## Results

A progressive increase in human plasma inorganic fluoride was observed during methoxyflurane anesthesia (fig. 1). Most control samples were below 1 μmol/l, the lower limit of detection. Increased fluoride ion was found at the first sampling interval (15 min); the highest recorded concentration was 48 μmol/l (4 hours). Concomitant increases in both plasma and urinary fluoride concentrations and fluoride excretion in one patient are shown in figure 2.

¶ Beckman Instruments, Fullerton, California.
** Beckman RC-16B2 conductivity meter and pipet-type cell (10 $cm^{-1}$).

TABLE 1. Inorganic Fluoride Concentrations in Plasma and Urine after Three Hours of Exposure to Methoxyflurane

| | Control* (μmol/l) | Methoxyflurane* (μmol/l) | Number of Animals |
|---|---|---|---|
| Mouse | | | |
| Plasma | 7 ± 1 | 113 ± 17 | 10 |
| Rat | | | |
| Plasma | <1† | 19 ± 2 | 10 |
| Urine | 60 ± 30 | 455 ± 19 | 10 |
| Guinea pig | | | |
| Plasma | 7 ± 1 | 55 ± 3 | 10 |
| Urine | 101 ± 18 | 563 ± 253 | 10 |
| Rabbit | | | |
| Plasma | 2 ± 0 | 45 ± 3 | 10 |
| Urine | 47 ± 7 | 213 ± 54 | 10 |
| Dog | | | |
| Plasma | 2 ± 0 | 85 ± 26 | 4 |
| Urine | 336 ± 9 | 5,875 ± 1,860 | 4 |
| Monkey | | | |
| Plasma | <1† | 26 ± 3 | 2 |
| Urine | 157 ± 124 | 1,085 ± 715 | 2 |

* Mean ± SE.
† The lower limit of detection was 1 μmol/l fluoride ion.

Concentrations of inorganic fluoride in human urine *during* and immediately after methoxyflurane anesthesia approached those obtained on the first postoperative day (fig. 3). Urinary fluoride ion concentrations before anesthesia were less than 90 μmol/l; levels as high as 3,500 μmol/l were recorded during anesthesia. Patients who received halothane had urinary fluoride concentrations consistently below 80 μmol/l.

Defluorination of methoxyflurane in all *animal* species anesthetized in the chamber was indicated by the marked increases in plasma and urinary inorganic fluoride (table 1). The ranges of absolute concentration increases in plasma and urinary fluoride were 18–106 μmol/l and 166–5,539 μmol/l, respectively; no correlation with either body weight or sex was observed.

As noted in figure 4, calf urinary and plasma inorganic fluoride also increased during administration of methoxyflurane.

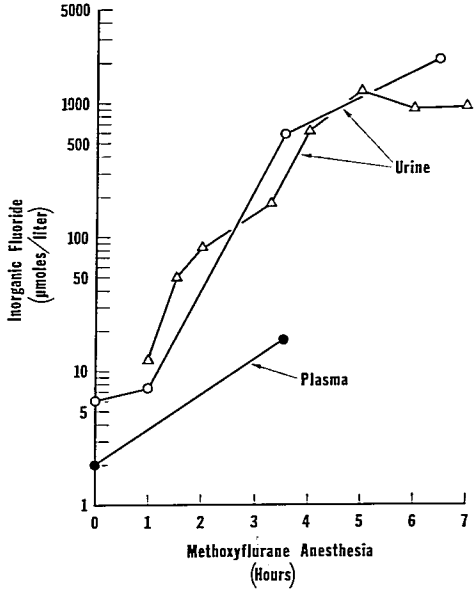


FIG. 4. Inorganic fluoride concentrations in calf urine and plasma during methoxyflurane anesthesia. Plasma and urine samples were collected simultaneously from one calf (circles); urine only was collected from another (triangles).

## Discussion

Since *all* seven species in the study metabolized methoxyflurane to fluoride ion, defluorination alone is not a sufficient criterion by which to select an animal model for further evaluation of inorganic fluoride as the causative factor of methoxyflurane-induced renal dysfunction. Such selection would require knowledge of a specific species' rate of defluorination and its renal sensitivity to fluoride ion. However, our data do not permit ranking of the species by rate of defluorination because the doses administered were adjusted to a pharmacologic end-point, *i.e.*, no response to a painful stimulus or surgery, and anesthetic uptake per animal was not measured. It is unfortunate that quantitative correlation of serum fluoride ion with renal dysfunction has been reported for only two species, dog and man. Changes in renal function have been observed in man at or above 106 μmol/l [11] and in the dog above 204 μmol/l.[14] Toxicity was not detected in either dog or man at concentrations of approximately 30 μmol/l.[14, 21] The present data show that hyperfluoremia in the dog after three hours of methoxyflurane anesthesia approaches levels known to be toxic in man. These studies were terminated after 3 hours, so it is not known whether the increase in fluoride ion in serum to 85 μmol/l represents the peak concentration in the dog, or whether deeper anesthesia and/or longer duration of exposure would achieve known toxic levels. As in man, there was a concomitant increase in urinary fluoride.

In view of the above human–dog analogy, and the documentation of methoxyflurane defluorination in the dog, this species is being studied to assess the quantitative relationship of inorganic fluoride to renal dysfunction during methoxyflurane anesthesia.

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## Drugs and Their Actions

**CATECHOLAMINE METABOLISM AND L-DOPA** Free and conjugated epinephrine and norepinephrine were relatively unaffected after 3 months of oral administration of L-dopa to 14 patients with Parkinson's disease, although urinary excretion of their most abundant metabolite, vanillylmandelic acid, was moderately increased. Large oral doses of L-dopa are needed to saturate intestinal dopa-decarboxylase to enable small amounts to reach target areas in the brain. Low levels of the drug associated with high levels of acidic biogenic amine metabolites in plasma indicated extensive catabolism of dopa in the intestine and other organs. Clinical improvement was related to CSF levels of the metabolite homovanillic acid, which was not detectable in the CSF of two patients who failed to respond. (*Hinterberger, H., and Andrews, C. J.: Catecholamine Metabolism during Oral Administration of Levodopa, Arch. Neurol. 26: 245–252, 1972.*)